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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12Q 1/68, C07H 21/04, G01N 33/53

A1

(11) International Publication Number: **WO 99/04042**

(43) International Publication Date: 28 January 1999 (28.01.99)

(21) International Application Number: PCT/US98/14782

(22) International Filing Date: 16 July 1998 (16.07.98)

(30) Priority Data:
60/052,655 16 July 1997 (16.07.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SYNTHETIC OLIGONUCLEOTIDE–PEPTIDE CONJUGATES AND METHODS OF USE

(57) Abstract

Compositions and methods for detecting or purifying multiple selected nucleic acid molecules are provided. The compositions comprise oligonucleotide–peptide conjugates (OPCs) and antibodies immunologically specific for the conjugates. The oligonucleotide moiety of each conjugate specifically hybridizes with a selected target nucleic acid molecule. The peptide moiety acts as an antigen for generation of a specific antibody that uniquely binds to its respective OPC. OPCs are advantageously used in groups, for detection or separation of multiple target nucleic acids in a single test sample, by virtue of the unique antigen/antibody interaction that enables differential detection or capture of the OPC. Methods and kits are provided for using the OPC systems of the invention.

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SYNTHETIC OLIGONUCLEOTIDE-PEPTIDE CONJUGATES AND METHODS OF USE

This invention claims priority to U.S. Provisional Application No. 60/052,655, filed July 16, 1997, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

This invention relates to the field of diagnostics and separations. More specifically, the invention provides novel synthetic oligonucleotide-peptide conjugates for use in detection of specific nucleic acid molecules and separation of such molecules from a heterogeneous mixture.

BACKGROUND OF THE INVENTION

The use of modified oligonucleotides as probes for detecting specific nucleic acid molecules has advanced the filed of DNA diagnostics in recent years. Detectably labeled oligonucleotides have been used to detect specific nucleic acids in a mixed population of nucleic acids and other biological molecules. This type of detection is currently performed with biotinylated DNA, digoxigenin-labeled nucleic acids and FITC-labeled DNA. In the cased of biotinylated DNA, the biotin is detected indirectly with streptavidin-labeled conjugates. Digoxigenin-labeled nucleic acids are detected with antibodies immunologically specific for digoxigenin. FITC acts as either a fluorochrome or an antigen.

A limitation to the biotin, digoxigenin and FITC detection systems is the lack of diversity among the labels. This prevents the use of multiple probes for hybridization assays because it is not possible to differentiate between two different probes that are labeled identically.

Thus, a need exists to develop a nucleic acid detection system that can provide a wide diversity among labels. It would be of further advantage to develop a system capable of separating or purifying target nucleic

acids from a mixed population of nucleic acids or other biological molecules. Compositions and methods comprising such a system would enable multiple nucleic acid detection and separation procedures, which heretofore have been limited or unavailable.

SUMMARY OF THE INVENTION

Provided in accordance with the present invention are compositions and methods that enable detection and/or separation of one or more selected nucleic acids in a test sample containing a larger population of nucleic acids or a heterogeneous mixture of nucleic acids and other molecules.

According to one aspect of the invention, a method is provided for determining the presence or quantity of one or more target nucleic acid molecules in a test sample suspected of containing one or more of the target nucleic acid molecules. The method comprises the steps of: (a) providing a set of oligonucleotide-peptide conjugates, each member of the set comprising (i) an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and (ii) a peptide moiety that binds to one of a set of antibodies, each member of the antibody set being immunologically specific for only one member of the oligonuclectide-peptide conjugate set; (b) contacting the test sample with the set of oligonucleotide-peptide conjugates under conditions that permit the specific hybridization of the oligonucleotide moieties to the one or more target nucleic acids, if present; (c) contacting the test sample with the antibodies, under conditions permitting binding of each member of the oligonucleotide-peptide conjugate set to its immunologically specific antibody, thereby forming a set of complexes, each complex comprising the target nucleic acid molecule, if present, one member of the set of oligonucleotide-peptide conjugates, and an antibody immunologically specific for that member; (d)

separating the complexes from the test sample; and (e) detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes, thereby determining the presence or quantity of each of the target nucleic acid molecules in the sample.

In a preferred embodiment of the aforementioned method, the step of contacting the test sample with the antibodies further comprises separately contacting an aliquot of the test sample with each member of the antibody set, thereby forming a complex in each aliquot comprising the target nucleic acid, if any, one member of the set of oligonucleotide-peptide conjugates, and the antibody immunologically specific for the member of the oligonucleotide-peptide conjugate set. In this embodiment it is particularly preferred that the respective antibodies are attached to a solid support and the step of contacting the test samples with the antibodies results in separately capturing each member of the set of complexes on the solid support.

In another embodiment of the aforementioned method, the target nucleic acids (or, alternatively, the oligonucleotide moieties of the set of oligonucleotide-peptide conjugates) are labeled with a reporter substance and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes is accomplished by detecting the reporter substance. In another embodiment, the selected target nucleic acids comprise RNA and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes is accomplished by contacting the complexes with a detectable antibody immunologically specific for DNA/RNA hybrid molecules.

In another embodiment of the aforementioned method, each member of the antibody set is labeled with a reporter substance unique to that member of the antibody set. The term "unique" means, for example, that if the antibody set has three members, each will be labeled with

a different reporter substance. The step of detecting the nucleic acid molecules, if any, associated with each member of the set of complexes is accomplished by detecting the presence or amount of each unique reporter substance in the set of separated complexes. It is preferred that the step of separating the complexes from the test sample comprises capturing the complexes on a solid support, and, optionally, thereafter releasing the captured complexes from the solid support.

According to another aspect of the invention, a method is provided for separating one or more target nucleic acid molecule from a test sample suspected of containing one or more of the target nucleic acid molecules. The method comprises the steps of: (a) providing a set of oligonucleotide-peptide conjugates, each member of the set comprising (i) an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and (ii) a peptide moiety that binds to one of a set of antibodies, each member of the antibody set being immunologically specific for only one member of the oligonucleotide-peptide conjugate set; (b) contacting the test sample with the set of oligonucleotide-peptide conjugates under conditions that result in the specific hybridization of the oligonucleotide moieties to the one or more target nucleic acids, if present; (c) separately contacting an aliquot of the test sample with each member of the antibody set, thereby forming a complex in each aliquot comprising the target nucleic acid, if any, one member of the set of oligonucleotide-peptide conjugates, and the antibody immunologically specific for the member of the oligonucleotide-peptide conjugate set; and (d) separating the complexes from each aliquot of the test sample. In one embodiment, this method further comprises releasing the target nucleic acids from the complexes.

The preferred practice of this method is wherein the antibodies are attached to a solid support

and the step of contacting the test samples with the antibodies results in separately capturing each member of the set of complexes on the solid support.

According to another aspect of the present invention, an assembly of compositions is provided for detecting one or more selected target nucleic acids in, or separating one or more selected target nucleic acids from, a test sample suspected of containing the one or more target nucleic acids. This assembly comprises (a) one or more oligoncleotide-peptide conjugates, each comprising a peptide moiety and an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and (b) one or more antibodies, each being immunologically specific for only one of the one or more oligonucleotide-peptide conjugates. In preferred embodiments, this assembly is prepared for detecting or separating two or three or more different nucleic acids.

According to yet another aspect of the invention, a test kit is provided for detecting one or more selected target nucleic acids in, or separating one or more selected target nucleic acids from, a test sample suspected of containing the one or more target nucleic acids. The kit comprises a container containing: (a) instructions for preparing one or more oligoncleotide-peptide conjugates, each comprising a peptide moiety and an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and (b) one or more antibodies, each being immunologically specific for only one of the one or more oligonucleotide-peptide conjugates. Alternatively, the kit contains the peptide moieties of the one or more oligonucleotide-peptide conjugates (preferably each bound to a solid support), and instructions for completing preparation of the conjugates. As another alternative, the kit may contain the one or more oligonucleotide-peptide conjugates fully prepared, instead of the instructions for preparing the conjugates. Such test kits also may include, optionally,

reagents for preparing the oligonucleotide-peptide conjugates, and instructions and reagents for using the conjugates and antibodies to detect or separate one or more target nucleic acids.

Additional features and advantages of the present invention will be better understood by reference to the detailed description and examples set forth below.

DETAILED DESCRIPTION OF THE INVENTION

Provided in accordance with the present invention is a system for 1) the detection of a multiplicity of specific sequences located within a target nucleic acid molecule (e.g., PCR product or non-amplified DNA or RNA), 2) the detection and differentiation of PCR products from a multiplex reaction, or 3) the separation of specific nucleic acids from a heterogeneous solution. These schemes rely on the application of a synthetic molecule, which is a combination molecule comprising an antigenic peptide moiety and an oligonucleotide moiety (the combination molecule is referred to hereinafter as an "OPC" - oligonucleotide-peptide conjugate). The oligonucleotide moiety is designed to specifically hybridize with a pre-determined target nucleic acid (DNA or RNA). The peptide moiety, either by itself, or in combination with adjuvants, carriers, or the oligonucleotide moiety, serves as an antigen for the production of immunologically specific antibodies. For purposes of this invention, the term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein of interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

Specifically exemplified because of the breadth of its utility, but not intended to be limiting, is the use of OPCs in a detection system. Persons skilled in the art will appreciate that the same underlying

principles enable the use of these conjugates for separating and purifying specific nucleic acid molecules.

As mentioned, this type of detection is currently performed with biotinylated nucleic acids (where the biotin is detected indirectly with streptavidin-labeled conjugates), digoxigenin labeled nucleic acids (where the digoxigenin serves as an antigen), and FITC labeled DNA (where the FITC can act as a fluorochrome or as an antigen). Those systems are limited by the lack of diversity among the labels, thereby preventing the use of multiple probes for solution based assays (i.e., it is impossible to differentiate between two different probes labeled identically).

Using oligonucleotide-peptide conjugates as probes, multiple probing can be accomplished since each probe has a unique label. For instance, PCR products generated from a primer set may have many potential sequence variations within the internal portion of the molecule (i.e., allelic variations, as with the HLA genes). Using the currently available technology, separate assays would have to be performed to detect each sequence variation. However, these variations can be differentiated by using a battery of oligonucleotide-peptide conjugates, each conjugate being designed to hybridize to a particular allele while possessing a unique antigenic marker. This has important applications in detection and diagnostic testing, as it enables high-throughput solution-based multiple screenings in a single test sample.

The sections below describe how to make and use the OPCs of the invention. Unless otherwise specified, general molecular biology procedures, such as those set forth in Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989) (hereinafter "Sambrook et al.") or Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1997) (hereinafter "Ausubel

et al.") and general DNA and peptide synthetic methods, such as those set forth in Echstein and Ekstein (eds) Oligonucleotides and Analogues: A Practical Approach, Oxford University Press (1992), or Merrifield & Steward, "Automated Peptide Synthesis", Nature 207: 522-523 (1965), or Thuong & Asseline, "Chemical Synthesis of Natural and Modified Oligonucleotides", Biochimie 67: 673-684 (1985) are used.

I. Design of OPCs

As described above, the system for using the OPCs of the invention revolves around a molecule in which the oligonucleotide is homologous to a DNA sequence to be detected and the peptide has been used as an antigen for the production of peptide antibodies. There is no limitation as to which oligonucleotides or peptides can be used in this conjugate, thus allowing tremendous flexibility and variety within this synthetic molecule. Any peptide which can be used to generate a specific antibody can be applied in this system, as well as any oligonucleotide which will specifically hybridize to a target DNA or RNA sequence. An antibody immunologically specific for the peptide portion of the conjugate, whether monoclonal or polyclonal, is an integral part of the system of the present invention. Such antibodies are produced by standard methods. Monoclonal antibodies may be prepared according to general methods of Köhler and Milstein, following standard protocols.

As mentioned, the advantage of the OPCs of the invention is that multiple detections or purifications (sometimes referred to herein as "multiplex reactions") can be achieved from a single test sample, or from a plurality of similar test samples (e.g., blood samples from several patients being tested for allelic anomalies in a particular gene). Accordingly, the invention contemplates generating a group or set of OPCs. The term "set" means, for purposes of this invention, two or three

or more OPCs. The term "set" is sometimes substituted with the terms "multiplicity", "plurality", "series" or "array", all of which are used interchangeably. It will be understood, however, that each OPC can be used individually in a manner similar to digoxigenin-based techniques, for example.

There is no upper limit to the number of OPCs that can be generated for a particular array. However, preferred for the practice of the present invention are multiples that are convenient for commercially available assay kits, such as 96-well microtiter plates. As another example in the biological testing field, a six-OPC array can be generated in which each OPC has a unique peptide attached to an oligonucleotide specific for DNA from a specific mycoplasma contaminant.

The oligonucleotide moiety of the OPC should be of a size sufficient to effect specific hybridization with the target nucleic acid, without an excess of "noise" or false positives. In this invention, "specific hybridization" means the association between two single-stranded nucleic acid molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence. Oligonucleotides between 10 and 100 bases in length are preferred for practice of the invention. Particularly preferred are oligonucleotides between 15 and 30 nucleotides, and most preferably 18-24 nucleotides long.

The peptide moiety should be of a size that is relatively convenient to prepare synthetically, though this is not an absolute requirement. Moreover, it must

be of a size to be antigenic, either alone or in combination with an adjuvant, or in a modified form (e.g., carrying protecting groups as discussed in greater detail below). Peptides between 6 and 30 residues in length are preferred for practice of the invention, and peptides most preferably are between 12 and 14 residues.

For practice of the present invention, it is preferred that peptides are generated which are strongly antigenic. However, weakly antigenic peptides may also be used, since numerous methods have been utilized for stimulating general immune responses for weakly antigenic substances. For example, adjuvants, such as complete Freund's and Ribi's, have long been used for this purpose. Another approach to stimulating the immunogenicity of a weakly-antigenic peptide or protein has been to couple the weak antigen to a carrier protein that is known to be a good immunogen. Common carrier proteins include keyhole limpet hemocyanin, fowl gamma-globulin and bovine serum albumin. Alternatively, the immunogencity of a weak antigen may be enhanced by polymerizing it into large aggregates by way of cross-linking agents, such as glutaraldehyde. In a similar method, solid-phase resins and peptide synthetic methods may be employed to synthesize a peptide repeatedly, to form a highly-branched complex.

As described in greater detail below, the oligonucleotide moiety and the peptide moiety can be synthesized together, or synthesized separately and linked together, in different orientations as follows:

5'-oligo-3' --- N terminus-peptide-C terminus
3'-oligo-5' --- N terminus-peptide-C terminus
base of oligo --- N terminus-peptide-C terminus
5'-oligo-3' --- C terminus-peptide-N terminus
3'-oligo-5' --- C terminus-peptide-N terminus
base of oligo --- C terminus-peptide-N terminus
5'-oligo-3' --- functional group on peptide (e.g., thiol)

3'-oligo-5' --- functional group on peptide

base of oligo --- functional group of peptide

Each of these linkages is contemplated for use in the present invention. It should be understood that the oligonucleotide moiety and the antigenic moiety must be functionally linked to one another. For purposes of the present invention, the term "functionally linked" is defined generally as linking of the moieties in such a way that each moiety retains its intended function.

II. Synthesis of OPCs

Regarding synthesis of the OPCs, presently both peptides and oligonucleotides are routinely synthesized, as well as a hybrid class of molecule, i.e., peptide nucleic acids (PNA). More recently chimeric molecules described as oligonucleotide-peptide conjugates have been synthesized for use as antisense therapeutics. The linkage between the oligonucleotide and peptide apparently can reduce the *in vivo* degradation rate of the oligonucleotide moiety. However, the application of oligonucleotide-peptide conjugates as described in accordance with the present invention heretofore has not been described.

The synthesis of a hybrid DNA/peptide molecule can be accomplished by several different approaches. For instance, the use of modified oligonucleotides as probes is very common and well documented.

One suitable location for the linkage between the oligonucleotide and peptide is the 5' or 3' ends of the oligonucleotide and an available amine, carboxy, or thiol on the peptide. The specific location of the linkage on the peptide will depend on the primary structure of that molecule. Suitable synthesis options are outlined below:

1. Organic Solvent Based/Protected Fragments

a. Continuous synthesis of DNA-Peptide on resin by solid phase synthesis;

b. Synthesis of protected DNA on resin followed by fragment coupling of protected peptide.

2. Native/Semi-Protected Synthesis

a. Solid phase coupling of DNA and peptide in aqueous solution;

b. Coupling of DNA and peptide in aqueous solution.

Two major approaches can be used to synthesize the chimeric molecule, i e., total synthesis and fragment coupling. With total synthesis, the oligonucleotide-peptide chimeric molecule is synthesized continuously on a solid phase. Fragment coupling involves separate synthesis of the DNA and peptide followed by coupling of the fragments. Of these two, the chemoselective ligation of peptide and oligonucleotide fragments is preferred.

Total Synthesis of a Chimeric Molecule. Synthesis of peptides and oligonucleotides take advantage of the solid phase principle. With careful choice of chemistry, it is feasible to synthesis the complete oligonucleotide-peptide chimeric by solid phase methodology. Peptide and DNA synthesis techniques can be merged in order to synthesize the hybrid molecule. This would allow for greater efficiency and yields of the oligonucleotide-peptide chimeric molecule (oligonucleotide-peptide conjugate). Two options are available for total synthesis: synthesis of DNA followed by peptide or peptide followed by DNA. Using a solid phase oligonucleotide with a 5′ primary amine, amino acids are coupled to the oligonucleotide by standard solid phase Fmoc chemistry. In this manner, the oligonucleotide is the support for peptide synthesis. Assuming the peptide (amide) backbone (this can be determined experimentally) is resistant to the deprotection conditions required to remove the pac-amidite protecting groups (Harlambidis et al., Nucl. Acids Res. 18: 493-499, 1990), the chemical strategy for peptide synthesis following oligonucleotide synthesis

will utilize Fmoc temporary protection with acid labile (TFA) groups for permanent protection (side chain). Two strategies for this approach are presented below.

In the first strategy, the oligonucleotide is synthesized using pac-amidites on a standard resin followed by amidating the 5' hydroxyl and extending the chain using carbodiimide, or carbodiimide/HOBt, or carbodiimide/active ester chemistry (e.g., NHS active esters). The protecting groups on the peptide portion are removed using TFA followed by deprotection and cleavage from the resin of the oligonucleotide-peptide chimeric using ammonia. If the oligonucleotide portion of the molecule suffers depurination as a result of TFA exposure, an alternative step is to build peptide portion with no side chain protection, therefore, no TFA treatment is necessary. Alternativley, only protecting groups for permanent side chain protection that will be removed by the final ammonia deprotection step (therefore no TFA required) are used. Examples of this would be the t-butyl ester protection for aspartic and glutamic acids and possible t-butyl ether protection for serine and threonine. Lysine would be a problem since the t-boc protection may be stable to the base conditions. Other protecting groups for lysine may be more appropriate, e.g., a trityl group or the like, which would only require dichloroacetic acid. It is known from synthesis of oligonucleotides that depurination does not occur with this reagent. Trityl protection is already available for several amino acids and could be used to protect lysine, histidine, and cysteine, arginine, glutamine, asparagine, and possibly serine, threonine, and tyrosine. If unavailable, synthesis should not be difficult.

If problems are encountered using the method described above, a more orthogonal (chemoselective) approach is used. Employing a base stable covalent bond linking the first nucleotide to the resin, the oligonucleotide is extended in the usual fashion,

subsequently amidated, and then deprotected. The peptide is then extended in the normal fashion using active esters to form the peptide bonds. Active esters are selective molecules and should not cross react with the deprotected bases on the oligonucleotide. This would obviate the need of exposing the peptide to the ammonia conditions of oligonucleotide deprotection. This method is intended for use only if it is determined that the peptide backbone does not survive the ammonia deprotection conditions utilized for oligonucleotides. Again, the same problems with side chain protection and TFA exposure, as discussed above, are relevant here, as are the solutions presented to those problems. The final step is removal of the oligonucleotide- peptide conjugate from the resin support. Here, a linker not requiring ammonia cleavage is necessary. There are many possibilities commercially available including photolysis with UV radiation of a photolytic handle or possibly a disulfide linkage.

It is an option to construct the peptide from amino acids requiring no side chain protection (ala, gly, ile, phe, etc.) or synthesize the peptide and leave side chain protecting groups in place. For the latter option, this would be practical if antibodies are raised against protected peptides and the protected peptides are soluble in aqueous solution.

Yet another option is to mix N-Fmoc strategy with Merrifield protecting groups (benzyl based side chain protection). The benzyl protecting groups are esters, and as such are labile to base hydrolysis. They may actually be an improvement when compared to the lability to ammonia hydrolysis of t-butyl protection used in the FMOC chemical strategy. Benzyl or butyl protected peptides are tested for base hydrolysis lability by mixing protected amino acid with ammonia (oligo deprotection conditions); analyze on reverse phase at 220 nm or following derivatization with PITC. It should be

noted that this combination of benzyl protecting groups and N-Fmoc synthesis yields a new type of amino acid synthesis process. Synthesizing the peptide first also requires building the peptide on a resin labile to base hydrolysis, such as the Merrifield Resin, where the linkage between peptide and resin is a benzyl ester. The peptide is constructed so that the only primary amine is limited to the N terminal amino acid. This primary amine acts as the reacting group for the coupling of activated nucleotide phosphoramidites in a standard DNA synthesis. In this manner, the peptide is synthesized followed by the direct addition of the protected nucleotides. An oligonucleotide with a peptide located on the 3′ end of the molecule results. Again, problems with TFA exposure and Ammonia deprotection would be the same as described above. Blocking the 3′ end of the oligonucleotide is useful for preventing mispriming if the conjugate is added to PCR reactions. In addition to a standard peptide, a multiple chain peptide can be used as an antigen, thus increasing the immunogenicity of the peptide and hence the signal. Furthermore, both the oligonucleotide-peptide conjugate combined can be used as an immunogen to further enhance antibody binding.

Synthesizing the peptide followed by DNA provides several additional options. In this methodology, the peptide requires protecting groups which can be cleaved by base hydrolysis. This is fundamentally important since the phosphoramidites used for DNA synthesis must be coupled to only one primary amine or hydroxyl on the peptide. Synthesis of the peptide, followed by acid deprotection, and then capping with acetic anhydride will eliminate all primary amines and hydroxyls. This strategy is practical if 1) the peptide is synthesized on a Merrifield resin with N-Fmoc chemistry, and 2) the side chain protecting groups are removed with TFA while the primary amine is still protected with the N-Fmoc group (assuming) the Fmoc group

is stable to TFA). Following deprotection, the primary amines and hydroxyls are capped. The N-Fmoc group is then removed and the DNA subsequently added to the N-terminal amine. In this approach, the capped peptide is used as the antigen for antibody synthesis. Another approach is to use an alternative to Fmoc for the terminal amino acid.

The continuous solid phase method is a practical method of synthesis for large scale production of the conjugate. Since researchers will presumably be synthesizing their own unique oligonucleotide probes, with the continuous solid phase technique it would be necessary to provide the peptide sequence for synthesis along with the corresponding antibody used for detection. Alternatively, a series of solid resins to which the respective peptides are attached, prepared for oligonucleotide synthesis thereon, is produced. The users of the OPCs simply follow instructions on initiation of oligonucleotide synthesis, thereby preparing OPCs customized in their laboratories for a particular assay or purification.

Solid Phase Chemoselective Ligation of Fragments. Coupling fragments where one fragment is immobilized on a solid phase possesses all the advantages of simplified sample handling associated with solid phase methods in general. Immobilizing DNA (either covalent or passive) to a resin allows for coupling of the peptide followed by efficient removal of unreacted peptide. The reverse is also true. One reason why the chemoselective ligation technique is preferred over the continuous (solid phase stepwise) approach is the already substantial body of work published in the last five years or so concerning the synthesis of multiple antigenic peptides (MAPs) using, among other things, chemoselective ligation of unprotected peptides. MAPs are synthetic proteins showing promise in both biochemical and biomedical applications. In the synthesis of MAPs,

unprotected peptide fragments are ligated to each other chemoselectively (that is, one specific product being formed exclusively). In the synthesis of oligonucleotide- peptide chimeric molecules, an unprotected peptide is ligated to an oligonucleotide probe.

Chemoselective ligation, also referred to as segment or fragment condensation, has been a topic for research in synthetic peptide chemistry for many years. The technique of constructing large peptides by ligating shorter, protected peptide fragments has not been particularly successful, partly due to the limited solubility of the protected peptide fragments. The use of unprotected peptides as ligation components, however, has improved the situation since these molecules are water soluble and coupling is conducted in aqueous solutions at high concentrations.

Another reason for the current success in aqueous ligation of peptides is the already substantial body of chemical techniques used for the chemoselective ligation reaction. Currently three types of chemistries have proven successful: a) thiol chemistry employing thioalkylation and thiol-disulfide exchange; b) weak base - aldehyde reactions forming aminal and imine type covalent linkages; and c) use of natural enzymes in reverse proteolysis reactions.

In the case of the oligonucleotide-peptide conjugates, the thiol and weak base chemistries appear to offer the most promise. Several methods are available for coupling, the first employing a bifunctional cross-linking reagent and the second involving direct activation of one of the fragments. The practical choices for peptide functional groups for coupling are the N-terminal amine, the lysine sidechain amine, the cysteine thiol, the carboxyl groups of glutamic and aspartic acids, and the terminal carboxyl group. For native DNA, the choices are limited to the 5′ hydroxyl or

5' phosphate, or following derivitization of nucleotide bases by the addition of reactive hydroyl, amine, carboxyl or thiol groups. However, synthetic oligonucleotides, which may also be modified as stated above, can be modified at both the 5' and 3' ends with the addition of amines, carboxyls and thiols. Modified oligonucleotide bases may also be added to the internal portion of the oligonucleotide, such as, but not limited to, 4-thio-uracil deoxyriboside, which can also serve as sites for peptide coupling These modified groups are normally attached to carbon linkers which will serve to separate the conjugated molecules. The simplest coupling of DNA and peptide will make use of either a homobifunctional (e.g., glutaraldehyde) or heterobifunctional cross-linker. With cross-linking, one of the molecules is mixed with excess linker and reacted with one end of the activated agent. A separation is required to remove the unreacted cross-linker from the activated molecule. That molecule is then coupled to the second half of the chimera. Due to the potential for peptide-peptide or DNA-DNA coupling, using a homobifunctional agent is least desirable. A heterobifunctional cross-linking reagent allows for only one conjugated product.

It is feasible and practical to activate either the DNA or peptide for the coupling. A free carboxyl group of the peptide can be modified to an active ester by treatment with carbodiimide/HOBt or carbodiimide and N-hydroxysuccinimide. Alternatively, a cyclic anhydride (succinic anhydride, for example) can be used to add a carboxy to the terminal amine (when dealing with peptide amides). It should be noted that a peptide will always contain a free c-terminal carboxyl group unless it is a peptide amide, $CONH_2$.

Thiol activation of a Cysteine side chain with 2,2'-dithiodipyridine in an aqueous buffer (phosphate, pH 5-6) yields an activated thiol. This is a chemoselective

active group specific for thiol groups. DNA in which a free thiol is added will then couple to the peptide containing the activated disulfide. This method is less energetic, but should be quite selective. Alternatively, the DNA thiol is activated and coupled to the peptide containing a free thiol. With cysteine-containing peptides, it is important to deprotect using TFA containing either ethanedithiol or mercaptoethanol to prevent thiol alkylation thus insuring the presence of free thiol in the deprotected peptide.

Table 1. Available functional groups and coupling methods.

DNA Group	Peptide Group	Coupling Method	Comment
Amino	Amino	DSG	Not directional
Amino	Thiol	SPDP	Chemoselective
Phosphate	Amino	EDC (carbodiimide)	
Thiol	Amino	SPDP	Chemoselective
Thiol	Thiol	DPDPB	Not directional
Amino	Carboxyl	EDC	
Thiol	Thiol	2,2'-dithiopyridine	Activated peptide or DNA
Amino	Carboxyl	EDC/N-Hydroxy succinimide	Activated Peptide

The first approach for ligation is to chemoselectively couple the unprotected peptide component (either C or N terminus) to the 5' end of the oligonucleotide. Chemoselectivity in this case simply means the peptide contains a nucleophile specific for an electrophile located at the 5' end of the oligonucleotide. The oligonucleotide-peptide conjugate is the only product formed because no oligo-oligo or peptide-peptide coupling is possible. This approach is preferable from a commercial perspective since the unprotected peptide, along with any ancillary reagents

required, could be supplied as a kit.

The peptide is added as a unit to either the hydroxyl, aminated, phosphorylated, or thiolyated 5′ end of the oligonucleotide. In this procedure, a bifunctional cross-linking reagent is reacted with the DNA and results in an activated 5′ thiol, amine, carboxylic acid, or aldehyde which can be used to couple to the peptide chemoselectively. For instance, a thiolated DNA can be linked to the peptide N terminal amine with the bifunctional cross-linker SPDP. Such an approach allows for the development of peptide antibody kits for the direct labeling of oligonucleotides. Alternatively, the immobilized DNA is first treated with the bifunctional cross-linker, washed to remove excess reagent, and then reacted with the peptide. Activating the immobilized molecule simplifies the removal of excess cross-linking reagent.

The deprotected peptide in solution is attached to a deprotected, resin-bound oligonucleotide, assuming the covalent bond connecting the oligonucleotide to the resin support is not base labile. The oligonucleotide-resin is deprotected, e.g., with ammonia, the unprotected peptide ligated to the deprotected oligonucleotide-resin, followed by removal of the oligonucleotide-peptide conjugate from the solid support. In this process, the peptide is not exposed to the ammonia, which is a desirable feature of the process. The disadvantage is that a solid support with a special linker (or handle) would have to be used for the oligonucleotide synthesis in place of the more traditional solid supports. Such a linker may comprises, for instance, a disulfide linkage with diimide coupling of the oligonucleotide and the peptide.

A post-synthetic approach is for the person utilizing the conjugate to synthesize the oligonucleotide, deprotect in the customary fashion, and then non-covalently (and reversibly) link the

oligonucleotide to a membrane or beaded polymer solid support. Both modified 5′ and 3′ ends, or modified bases, of the oligonucleotide can be made available for ligation, and the unprotected peptide is then chemoselectively ligated to the bound oligonucleotide. The oligonucleotide-peptide conjugate is then released from the solid support. An exemplary protocol for the above strategy is:

1. adsorb DNA to a Qiagen column in Buffer A, the peptide remains in solution;

2. add cross linker or have activated DNA or peptide; perform the coupling and wash away uncoupled peptide;

3. switch buffers so the peptide sticks and uncoupled DNA can be washed away;

4. elute the chimeric molecule.

In many ways, immobilizing the peptide for the ligation could be a practical approach to conjugate formation. As mentioned above, the user of the conjugate synthesizes an oligonucleotide probe in the customary way with the final step being attachment of a functional group to the oligonucleotide (e.g., NH2, SH, etc.), which is necessary for ligation to the peptide. The unprotected peptide is provided either covalently bound to a resin, or passively adsorbed to a stationary phase. The oligonucleotide probe, deprotecting in the usual fashion, is then ligated to the unprotected peptide. After ligation the oligonucleotide-peptide conjugate is eluted from the matrix under mild conditions.

Solution Based Coupling. The solution based coupling utilizes all of the chemistries outlined above, but does not involve solid phase methodology. Concentration is not a problem since both peptide and oligonucleotide are both readily soluble in the aqueous buffers. The only reason to use solid phase methodology is the improved separation of unreacted materials. If these sample handling features of solid phase coupling

are not necessary, then coupling in free solution is an alternative. The characteristics of solution synthesis are: (1) the coupling reaction occurs in a homogeneous solution; (2) the molecules are coupled using same chemistries as solid phase methods; and (3) separation of the chimeric molecules from unreacted materials is required. Though solution based coupling is a viable alternative for practicing the present invention, solid phase coupling of fragments will likely be more attractive to most practitioners of the invention, due to the simplified means for obtaining the conjugates.

III. Using the OPC System:

As mentioned above, the OPC system comprises a plurality of OPCs, for use in detecting multiple nucleic acid targets in, and/or separating (sometimes referred to herein as "capturing") such targets from, a heterogenous mixture of biological or other molecules (e.g., biological fluids, water or food samples, and the like).

Referring to detecting, the term "detection means", sometimes used interchangeably with the terms "label", "detectable label" or "reporter substance" refers to any substance whose detection or measurement, either directly or indirectly, by a physical or chemical means, is indicative of the presence of an OPC complex. Examples of useful reporter substances include, but are not limited to: molecules or ions directly or indirectly detectable based on light absorbance ("chromophores"), fluorescence ("fluorophores"), reflectance, light scatter, phosphorescence or luminescence properties; molecules or ions detectable by their radioactivity properties; and molecules or ions detectable by their nuclear magnetic resonance or paramagnetic properties; and molecules detectable by their enzymatic properties.

Referring to capturing or separating, in some embodiments this is facilitated by immobilizing a capture agent on a solid support. As described in greater detail

below, the capture agent can be the specific anti-OPC antibody. In other embodiments, the capture agent may comprise streptavidin, protein A, or another antibody, such as an anti-DNA/RNA hybrid antibody. As use herein the term "solid support" can also mean an insoluble fluid support. Examples of solid supports useful for the practice of the present invention include, but are not limited to: membranes such as nitrocellulose, polystyrene or nylon membranes; microbeads or microparticles, such as agarose or latex beads, or magnetic or paramagnetic beads; and magnetic or paramagnetic fluids.

A series of OPCs is designed, each member having a different oligonucleotide and peptide component, respectively. Optionally, the oligonucleotide component of the OPC is made detectable or capturable (e.g. by labeling with biotin, which can be detected or captured *via* its interaction with streptavidin). Alternatively, the population of nucleic acid molecules containing (or suspected of containing the target) can be made detectable or capturable.

Antibodies that immunospecifically bind to each OPC in the series are generated. These can be used to detect or capture the OPC in an OPC-specific manner. Thus, each OPC is detectable or capturable by either the oligonucleotide moiety or the peptide moiety. However, it is the peptide moiety that gives the OPC its specific nature, inasmuch as a matching OPC-specific antibody is made for each different member of the group.

The system is used as follows:

1. A test sample containing (or suspected of containing) one or more of the nucleic acids to which one of the OPC series hybridizes is provided.

2. The series of OPCs is added to the test sample(s), under conditions that permit the specific hybridization between the OPCs and their respective target nucleic acids, if present.

3. The OPCs are contacted with the series of antibodies immunologically specific for the respective members of the OPC series, under conditions permitting the immunological reaction to occur. Steps 2 and 3 can occur in either order, but that the result is a three-part complex comprising the target nucleic acid, the OPC, and its respective antibody.

4. The nucleic acid (NA) /OPC/antibody complexes are then separated, or detected, or both, as described below.

In a preferred embodiment, the OPC antibodies are immobilized on a solid support (e.g., each different antibody is bound to a well of a microtiter plate), such that Step 3 above results in capture and sequestration of respective OPCs in the series. The presence or amount of OPC complex thus immobilized and sequestered may then be determined. Table 2 below sets forth several preferred ways of detecting the captured nucleic acids.

Table 2. Approaches to Detecting Selected Nucleic Acids

Selected Nucleic Acid	Label	Detection	Comments
PCR Product	Biotinylated Primer	Streptavidin Conjugates	Biotin is added to primer during synthesis
	Biotinylated Primer	Streptavidin Conjugates	Label is incorporated during polymerization steps
RNA	DNA/RNA Hybrid	Anti-DNA/RNA Antibody Conjugate	Such antibodies are available
Specific Nucleic Acid	Biotinylated Probe	Streptavidin Conjugate	A separate labeled probe is hybridized to a common sequence on the selected molecule

Using one of these approaches to detect selected nucleic acids, the steps of the method would be: (1) hybridize the OPCs to the respective targets; (2) immunospecifically capture the hybridized OPCs; and (3) detect the selected targets via one of the labels described above.

It will be appreciated that the nucleic acid detection means set forth in Table 2 can also be used as a capture means. Accordingly, in another embodiment of the invention, the system could be used as follows: (1) hybridize the OPCs to the respective targets; (2) capture the entire population of targets (i.e. as a way to separate the nucleic acids from other types of molecules in the sample); (3) optionally, release the population of targets; and (4) differentially label individual targets by immunospecifically binding the respective antibodies, each of which has been differentially labeled, e.g., with a unique reporter substance, such as a chromophore or fluorophore. Steps 1, 2 and 3 could be performed in any order. The advantage of this method is that the end product is a solution of OPC complexes, each hybridized to a different target and labeled with a unique label. A single sample of the solution could be assessed for the presence or amount of a selected target nucleic acid; e.g., spectrophotometrically or spectroflurometrically at the appropriate wavelengths for the chromophores or fluorophores used.

In some instances, it may be desirable to perform the above procedure without utilizing a capture agent for the population of targets. For instance, a mixed population of nucleic acid/OPC/antibody complexes can be immunoprecipitated, then resuspended and measured as described above.

IV. Test Kits and Other Applications

The OPC arrays of the present invention can be packaged into a variety of test kits, for use in

detection and purification of nucleic acids, or for therapy in delivering certain nucleic acids. Described below are several preferred embodiments for test kits.

At minimum, a test kit will comprise (1) the relevant series of peptide antigens, or instructions (i.e., sequence information) for synthesizing the antigens; and (2) the respective immunologically specific antibodies. The antibodies provided in the kit are used for the detection or purification. In a preferred embodiment, the antibodies are provided immobilized to a substrate, so that the OPC/target nucleic acid complex can be affinity captured. Alternatively, the antibodies will be provided already differentially labeled, e.g., with a series of chromophores or fluorophores.

As one example of a test kit, in a situation where a continuous synthesis of the conjugate, starting with the oliognucleotide, is to be performed, the test kit will contain the antibodies (optionally labeled or immobilized on a solid support) and sequence information for the array of peptide antigens and instructions on how to conduct the synthesis. The user of the OPC will design appropriate oligonucleotides for a selected separation or detection and will synthesize the entire set of OPCs according to the instructions provided.

As another example, in a situation where a continuous synthesis of the conjugate, starting with the peptide moiety, is to be performed, the test kit contains the antibodies and either (1) instructions and sequence information for the peptides or (2) the peptides themselves, immobilized on a resin suitable for continued synthesis of the oligonucleotide moiety. Again, the user of the OPCs designs appropriate oligonucleotides for a selected separation or detection.

As another example, where the oligonucleotide and peptide moieties are synthesized separately, then functionally linked, the kit may contain the antibodies and (1) the pre-made peptides, either in solution or on a

solid support; and (2) the linking agent and instructions for use. Again, the user designs and synthesizes the oligonucleotides, then links them to the peptides according to the instructions provided in the kit.

As another example, in clinical diagnostic situations where an assay for a pre-determined set of nucleic acid targets is performed repeatedly (e.g., diagnostic for allelic anomalies associated with a particular disease), the kit contains the antibodies and a set of ready-made OPCs each with a unique peptide moiety linked to an oligonucleotide probe designed to identify a particular target nucleic acid (e.g. an established allelic anomaly).

The test kits described above are simply examples of the many ways the OPC system of the invention can be designed and used for a variety of diagnostic, separatory, or even therapeutic applications. Like many molecular tools, OPCs will find numerous applications in addition to those described above and exemplified below. For instance, OPCs can be used as primers for PCR, and products which are amplified from OPC primers can be affinity purified using an immobilized antibody column, much the same as any antigen. This could be very useful in isolating differential display products and expressed sequence tags. Furthermore, the conjugate will also find use as an anti-sense therapeutic with the peptide serving as a ligand for a specific cell based receptor. Binding of the ligand to the receptor may be a means of targeting a specific antisense oligonucleotide for a given cell. If the chimeric oligonucleotide-peptide is linked with a disulfide bond, release of the oligonucleotide would occur up assimilation of the molecule into the reduced cellular environment.

The following examples are provided to describe the invention in greater detail. They are intended to illustrate various embodiments of the invention, and not to limit the invention in any way.

Example 1
The Detection of Specific Alleles
Located within a PCR Product

The analysis of genetic variation is fundamental to many diagnostic tests, including cystic fibrosis, HLA, and Alzheimer's testing. Often PCR can be performed on specific regions of DNA with the primer set being designed to flank and amplify the allelic variations. In this manner, one conserved primer set can amplify many or all of the genetic variations of a gene. The region located within the PCR product, i.e., between the primers, is the target for the analysis. By performing the PCR reaction with one biotinylated primer, e.g., the forward primer, the plus strand of the product can be labeled. (This 5' biotinylation is a very common technique used to label PCR products.) Though this discussion will examine biotinylated PCR products, other labels are available as well, including digoxigenin and fluorescent tags, such as FITC.

To detect specific variations within the PCR product, a set of oligonucleotide-peptide conjugates (with associated peptide antibodies) are designed to hybridize specifically to the variations of the biotinylated PCR strand. Each oligonucleotide-peptide conjugate will have specificity to a particular allele while possessing the unique antigen used to raise a specific peptide antibody. By mixing this set of oligonucleotide-peptide conjugates with the PCR product, followed by denaturing and annealing, the oligonucleotide- peptide conjugates will hybridize to their homologous sequences, i.e., if those sequences are present, and indirectly label those sequences with a unique antigen. If homology exists between the PCR product and oligonucleotide- peptide conjugate, then the resulting hybrid will possess a biotinylated plus strand hybridized to a oligonucleotide which is linked to a specific antigenic marker. Consequently, each sequence variation in the PCR product will be associated with a

specific antigen.

Several strategies and formats can be used to detect this biotin-PCR product/oligonucleotide-peptide conjugate complex. One approach could simply involve adsorbing each peptide antibody to a well of a microtiter plate, so to make each well a specific immunoassay for a specific nucleic acid sequence. The biotin-PCR product/oligonucleotide-peptide conjugate complex would be added to each well, where the complex would bind to the immobilized antibodies. The complex could be detected in a standard enzyme immunoassay format in which a streptavidin- enzyme is used to detect the biotin label. This approach has the added benefit of being a solution based hybridization which is more efficient than hybridizing nucleic acids when one strand is immobilized, as with a blot. An alternative method may involve capturing the biotinylated strand with immobilized streptavidin and then adding differentially labeled antibodies. This would be particularly applicable to antibodies differentially labeled with fluorescent tags, with each fluorophor having a distinct emission.

Example 2
Detection of Pathogenic Microorganisms with PCR and a Dipstick Assay

There has been tremendous focus in the past several years on the detection of pathogenic microorganisms, especially in food products. The answers to the detection needs have come in the form of expensive instrumentation and assays. For example, the DuPont subsidiary Qualicon was founded on a device which amplifies the ribosomal RNA of selected bacteria (e.g., E. coli O157) and then profiles the DNA pattern electrophoretically. Applied Biosystems has approached this topic by developing the TaqMan system which yields fluorescence as PCR product accumulates. Though both systems have proved useful, the instrumentation cost of these units alone reach well above $100,000 while the

consumable costs are proportionally high as well. A desirable assay for pathogenic microbes would be cheap, simple, and robust. The following is an alternative approach.

Magnetic beads with antibodies against pathogenic microorganisms are mixed with a test sample and vortexed. Using a magnet, the beads are pulled from the solution. The beads are washed to remove residual debris, suspended in a reverse transcriptase buffer, and heated to 95°C for 10 min. This heating step will lyse bacteria bound to the beads, thus releasing RNA molecules. In addition to the reagents for cDNA synthesis, the RT buffer contains primers sets which are all labeled with a single peptide antigen and which are also specific for the microorganisms under investigation. Each primer set is targeted to an identifying sequence within the associated genome of that organism. These primers set will be used for both cDNA synthesis and PCR. Upon cooling the mixture, the beads and solution are separated using a magnet, with the solution being transferred to a PCR tube.

Reverse transcriptase is added to the solution, followed by an incubation during which cDNA is synthesized. The solution is then adjusted for PCR by the addition of buffers, dNTPs, and Taq polymerase. Following the PCR, the tubes are opened and a solution of EDTA and oligonucleotide- peptide conjugated probes are added. These OPC probes are targeted against sequences located within the PCR product. The tube is sealed, heated to denature the PCR products, and then cooled to an annealing temperature suitable for the hybridization of the OPC probes. The addition of the EDTA is to chelate Mg ions and thus prevent the continued activity of the Taq polymerase.

The solution is added to a small glass tube containing a lyophilized antibody against the peptide label on the primers. This antibody is coupled to

colored latex microbeads. The antigenic PCR products and residual OPC primers are allowed to bind with the antibody-latex bead conjugate. A dipstick with the sequence specific anti-OPC antibodies immobilized to distinct locations is then added to the glass tube. With dipstick assays, a wick draws the test solution up the dipstick. As the antigens flow past the immobilized antibodies, the latex bead-PCR-OPC probes bind to their specific antibodies. As the complexes concentrate on the immobilized antibodies, the latex beads produce a visible color. This type of dipstick immunoassay is well documented and has been used to detect and differentiate numerous antigens in a simple low cost approach.

A modification of this system could involve labeling the primer sets differentially. In place of the uniform peptide labeled primers, each primer set could contain a common label (e.g., biotin) which will bind to the antibody-latex bead conjugate, and a peptide label specific for that PCR product. In this manner, several steps of the assay could be eliminated, however the verification of the PCR product by an internal probe would be lost. Though these examples make use of PCR (for signal enhancement), the use of sensitive detection methodologies could eliminate the amplification step. For assays in which the starting concentration of nucleic acids is relatively high, a direct detection is possible.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A method for determining the presence or quantity of one or more target nucleic acid molecule comprising a selected subpopulation of nucleic acids in a test sample suspected of containing one or more of the target nucleic acid molecules, the method comprising the steps of:

a) providing a set of oligonucleotide-peptide conjugates, each member of the set comprising:

i) an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules of the selected subpopulation; and

ii) a peptide moiety that binds to one of a set of antibodies, each member of the antibody set being immunologically specific for only one member of the oligonucleotide-peptide conjugate set;

b) contacting the test sample with the set of oligonucleotide-peptide conjugates under conditions that permit the specific hybridization of the oligonucleotide moieties to the one or more target nucleic acids, if present;

c) contacting the test sample with the antibodies, under conditions permitting binding of each member of the oligonucleotide-peptide conjugate set to its immunologically specific antibody, thereby forming a set of complexes, each complex comprising the target nucleic acid molecule, if present, one member of the set of oligonucleotide-peptide conjugates, and an antibody immunologically specific for the member;

d) separating the complexes from the test sample; and

e) detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes, thereby determining the presence or quantity of each of the one or more target nucleic acid molecule comprising the selected subpopulation of nucleic acids in the test sample.

2. The method of claim 1, wherein the selected subpopulation comprises allelic variants of a gene, and each target nucleic acid comprises a sequence uniquely associated with one of the variant.

3. The method of claim 1, wherein the selected subpopulation comprises mutations of a gene, and each target nucleic acid comprises a sequence uniquely associated with one of the mutations.

4. The method of claim 1, wherein the selected subpopulation comprises genomes of a pre-determined group of microorganisms suspected of being present in the test sample, and each target nucleic acid comprises a sequence uniquely associated with one of the microorganisms.

5. The method of claim 1, wherein the selected subpopulation comprises nucleic acid molecules encoding a pre-determined group of polypeptides suspected of being present in the test sample, and each target nucleic acid comprises a sequence uniquely associated with one of the polypeptide-encoding nucleic acid molecules.

6. The method of claim 1, wherein the step of contacting the test sample with the antibodies further comprises separately contacting an aliquot of the test sample with each member of the antibody set, thereby forming a complex in each aliquot comprising the target nucleic acid, if any, one member of the set of oligonucleotide-peptide conjugates, and the antibody immunologically specific for the member of the oligonucleotide-peptide conjugate set.

7. The method of claim 6, wherein the antibodies are attached to a solid support and the step of contacting the test samples with the antibodies results in separately capturing each member of the set of

complexes on the solid support.

8. The method of claim 6, wherein the selected subpopulation of nucleic acids is labeled with a reporter substance and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes is accomplished by detecting the reporter substance.

9. The method of claim 6, wherein the oligonucleotide moieties of the set of oligonucleotide-peptide conjugates are labeled with a reporter substance, and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes is accomplished by detecting the reporter substance.

10. The method of claim 6, wherein the selected subpopulation of nucleic acids comprises RNA and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of separated complexes is accomplished by contacting the complexes with a detectable antibody immunologically specific for DNA/RNA hybrid molecules.

11. The method of claim 1, wherein each member of the antibody set is labeled with a reporter substance unique to that member of the antibody set, and the step of detecting the nucleic acid molecules, if any, associated with each member of the set of complexes is accomplished by detecting the presence or amount of each unique reporter substance in the set of separated complexes.

12. The method of claim 11, wherein the step of separating the complexes from the test sample comprises capturing the complexes on a solid support.

13. The method of claim 11, which further comprises releasing the captured complexes from the solid support.

14. The method of claim 12, wherein the capturing is accomplished by biotinylating the selected subpopulation of target nucleic acids or the oligonucleotide moieties of the oligonucleotide-peptide conjugates and contacting the complexes with streptavidin immobilized on the solid support.

15. A method for separating one or more target nucleic acid molecule comprising a selected subpopulation of nucleic acids from a test sample suspected of containing one or more of the target nucleic acid molecules, the method comprising the steps of:

a) providing a set of oligonucleotide-peptide conjugates, each member of the set comprising:

i) an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules of the selected subpopulation; and

ii) a peptide moiety that binds to one of a set of antibodies, each member of the antibody set being immunologically specific for only one member of the oligonucleotide-peptide conjugate set;

b) contacting the test sample with the set of oligonucleotide-peptide conjugates under conditions that result in the specific hybridization of the oligonucleotide moieties to the one or more target nucleic acids, if present;

c) separately contacting an aliquot of the test sample with each member of the antibody set, thereby forming a complex in each aliquot comprising the target nucleic acid, if any, one member of the set of oligonucleotide-peptide conjugates, and the antibody immunologically specific for the member of the oligonucleotide-peptide conjugate set; and

d) separating the complexes from each aliquot of the test sample.

16. The method of claim 15, which further comprises releasing the target nucleic acids from the complexes.

17. The method of claim 15, wherein the antibodies are attached to a solid support and the step of contacting the test samples with the antibodies results in separately capturing each member of the set of complexes on the solid support.

18. An assembly of compositions for detecting one or more selected target nucleic acids in, or separating one or more selected target nucleic acids from, a test sample suspected of containing the one or more target nucleic acids, the assembly comprising:

a) one or more oligoncleotide-peptide conjugates, each comprising a peptide moiety and an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and

b) one or more antibodies, each being immunologically specific for only one of the one or more oligonucleotide-peptide conjugates.

19. The assembly of claim 18, for detecting or separating two or more different target nucleic acids.

20. The assembly of claim 18, for detecting or separating three or more different target nucleic acids.

21. A test kit for detecting one or more selected target nucleic acids in, or separating one or more selected target nucleic acids from, a test sample suspected of containing the one or more target nucleic acids, the kit comprising a container containing:

a) instructions for preparing one or more oligoncleotide-peptide conjugates, each comprising a peptide moiety and an oligonucleotide moiety that specifically hybridizes to one of the target nucleic acid molecules; and

b) one or more antibodies, each being immunologically specific for only one of the one or more oligonucleotide-peptide conjugates.

22. The test kit of claim 21, in which the container contains the peptide moieties of the one or more oligonucleotide-peptide conjugates and instructions for completing preparation of the conjugates.

23. The test kit of claim 21, in which the container contains the one or more oligonucleotide-peptide conjugates instead of the instructions for preparing the conjugates.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14782

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68; C07H 21/04; G01N 33/53
US CL :435/6, 7.1; 536/23.1, 24.3, 25.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1; 536/23.1, 24.3, 25.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, MEDLINE, CA, DERWENT
search terms: oligonucleotide, peptide, conjugate, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,525,465 A (HARALAMBIDIS et al.) 11 June 1996, col. 2, lines 6-10; col. 3, lines 44-55; col. 7, lines 43-55; column 8, lines 5-9.	1-23
Y	US 5,470,705 A (GROSSMAN et al.) 28 November 1995, col. 2, lines 34-50.	1-23
A	US 5,604,097 A (BRENNER) 18 February 1997, col. 11, lines 22-34.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
16 SEPTEMBER 1998

Date of mailing of the international search report
21 OCT 1998

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